Note

Spectral Properties of Acid Denaturation of Rat Liver Arginase

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As was shown in the previous paper, the acidification of a rat liver arginase (EC 3.5.3.1) solution denatured the enzyme.¹⁾ The denatured arginase was not able to recover its activity after direct transfer to alkaline assay media, but was able to recover it in the alkaline assay media after a short incubation at neutral pH. A similar result has been obtained with rabbit liver arginase.²⁾ This paper reports the secondary and tertiary structural changes of the enzyme as the pH decreases to denature the enzyme.

Rat liver arginase was purified according to the Hirsch-Kolb and Greenberg method.³⁾ To concentrated enzyme fractions $MnCl_2$ was added to a final concentration of 1 mM. After dialysis against distilled water overnight, the enzyme solution was lyophilized. The enzyme was dissolved in distilled water and filtered through a membrane filter. The protein concentration was determined from UV absorption with an absorption coefficient of 1.2 at 280 nm (1 mg/ml)⁴⁾ and a molecular weight of 118,000.³⁾

Acidification of the enzyme solution was done by the addition of a buffer solution of a desired pH.

CD spectra in the ultraviolet region (200~240 nm) were obtained with a JASCO model J-20 recording spectropolarimeter at 10°C (Fig. 1). The concentration of arginase was 2.39×10^{-7} M. Little conformational changes occurred in the protein on varying the pH between 7.2 and 4.1. The α -helix content within this pH range was almost constant at about 50%, as calculated according to the Greenfield and Fasman method.⁵⁾ A weak tendency of enhancement of the negative CD band at 217 nm, a measure of the β -structure content, might have been seen as the pH decreased from 7.2 to 4.1. But it was uncertain whether this tendency was significant because the CD band dependent on the β -structure was considered to be influenced by many factors including the nature of the solvents.^{5,6)} When the pH of the arginase solution was reduced to below 4.1, the regular structures were largely destroyed, and the α -helix content was reduced to 39.6% at pH2. And the enhancement of the negative band around 200 nm indicated that the content of random coils had increased.

Second derivative spectra were recorded with a Hitachi model 200 recording spectrophotometer equipped with an electronic derivative accessory at 5°C. The concentration of arginase was 8.06×10^{-7} M. The second derivative spectra of rat liver arginase at various pHs (Fig. 2) indicated that there was a critical point around pH 4.1, relating to the extent of the exposure of hydrophobic regions to the environmental solvent. The peak-to-peak distances, $\overline{CD}$ and $\overline{AB}$, indicated in Fig. 2, reflected the environmental states around phenylalanyl and tryptophyl residues, respectively. The environment around the phenylalanyl residues was unaltered at pHs above 4.1, but below this pH changed to be more polar. The state of existence of tryptophyl residues also changed

FIG. 1. Far Ultraviolet CD Spectra of Arginase at Various pHs.

Buffers used on the left were (—) pH 7.2, 10 mM PIPES; (---) pH 4.6, 10 mM acetate; (.....) pH 4.1, 10 mM acetate; and on the right, (—) pH 7.2, 10 mM PIPES; (.....) pH 3.5, 10 mM glycine; (---) pH 2, glycine.

FIG. 2. Second Derivative Spectra of Arginase at Various pHs.

The buffer systems were: pH 6.8, 50 mM Tris-acetate; pH 4.4–3.6, 50 mM acetate-HCl; pH 2, 50 mM glycine-HCl.

to be less hydrophobic as the pH decreased, and the changes appeared much clearer at pHs lower than 4.1. The tryptophyl minimum band also shifted from 291 to 289 nm in this pH region.

The capacity of rat liver arginase for binding 8-anilidonaphthalene-1-sulfonate (ANS) was determined fluorometrically in solutions at various pHs (Fig. 3) with a Hitachi 204 recording spectrofluorometer at 10°C. The concentration of arginase was 4.55×10^{-7} M and that of ANS was 1.61×10^{-6} M. The fluorescence intensities at 500 nm, excited at 365 nm, were recorded. After 30 min, the fluorescence intensities were measured at the emission maxima excited at 365 nm. As the pH decreased, the interaction between ANS molecules and hydrophobic regions increased gradually and around pH 4.1 increased sharply and then decreased. The hydrophobic regions, which were formed in the contact sites between the subunits, were exposed to the solvent and interacted with ANS, at least immediately after the dissociation.

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FIG. 3. The Binding of ANS to Arginase on Acid Denaturation.

Arginase solution (166.5 μ g protein in 5 mM MnCl_2 , the pH adjusted to 7.1 with a trace of KHCO_3) was mixed with ANS solution buffered at the indicated pH. (Left) the time-course; (right) fluorescence intensities at the emission maxima. Buffers were: 50 mM Tris-HCl in the pH range from 9 to 7.5, 50 mM Tris-acetate in the pH range from 6.9 to 5.6, 50 mM acetate-HCl in the pH range 5 to 4.1, and 50 mM glycine-HCl in the pH range from 3 to 2.

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